EXBHIBIT A

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Effect of High-Carbohydrate–Low-Fat Diets on Plasma Glucose, Insulin and Lipid Responses in Hypertriglyceridemic Humans

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Two levels of dietary carbohydrate (40% and 60% of calories) were incorporated into typical US diets and fed for 15 days each to eight patients with endogenous hypertriglyceridemia. Fasting blood samples were drawn on days 13, 14, and 15 of each dietary period, and analyzed for glucose, insulin, cholesterol, and triglyceride concentrations, as well as for triglyceride and cholesterol content of the various lipoprotein classes. In addition, these same measurements were made before and for three hours after the noon meal on days 14 and 15. Fasting plasma triglyceride (TG) and very-low-density lipoprotein (VLDL)-TG concentrations were significantly increased ($P < 0.005$) on the low-fat–high-carbohydrate diet. In addition, integrated postprandial insulin, TG, and VLDL-TG responses to the noon meal were significantly ($P < 0.01$–0.001) elevated on the low-fat–high-carbohydrate diet. No dietary-induced changes were noted in either the fasting or postprandial values of glucose cholesterol, chylomicron-TG, low-density lipoprotein-cholesterol, high-density lipoprotein (HDL)-cholesterol, HDL_2-cholesterol, or HDL_3-cholesterol. These results indicate that low-fat–high-carbohydrate diets accentuate the metabolic risk factors for coronary artery disease that are already present in patients with endogenous hypertriglyceridemia.

PREVIOUS RESULTS have indicated that low-fat–high-carbohydrate liquid formula diets can lead to postprandial hyperglycemia, hyperinsulinemia, elevated fasting plasma triglyceride (TG) levels, and reduced high-density lipoprotein (HDL)-cholesterol concentrations.[1-3] Since all of these changes have been shown to increase the risk of developing atherosclerotic heart disease (ASHD) in prospective studies,[4-9] these results are of obvious interest in light of dietary advice advocating the use of low-fat–high-carbohydrate diets in order to reduce the risk of ASHD.[10-12] More recently, it has been demonstrated that normal volunteers fed low-fat–high-carbohydrate solid food diets had significant increases in both postprandial insulin and fasting TG concentrations, associated with a decreased plasma HDL-cholesterol level.[13] These new observations reinforced concern that ingestion of low-fat–high-carbohydrate diets might have potentially harmful metabolic effects, and indicate that these changes can occur in response to ingestion of conventional, solid food diets. Given these findings, it was thought important to extend these observations to individuals who already are at an increased risk of developing ASHD. For this purpose, individuals with endogenous hypertriglyceridemia, without other known diseases were selected. In addition to increased plasma TG concentrations, such individuals have postprandial hyperglycemia,[14] hyperinsulinemia,[14,15] and low HDL-cholesterol levels.[16] Thus, they might be uniquely susceptible to the untoward effects of low-fat–high-carbohydrate diets. The results presented here confirm this suspicion.

MATERIALS AND METHODS

Eight volunteers (four men and four women) were selected based upon the presence of a plasma TG concentration between 200 to 400 mg/dL and a plasma cholesterol concentration less than 300 mg/dL. The mean (±SEM) plasma TG concentration on ad libitum diets in these subjects was 286 ± 16 mg/dL (range 239–353 mg/dL). Patients with diabetes mellitus were excluded on the basis of an oral glucose tolerance test.[17] Subjects with other diseases or those taking medications known to affect carbohydrate or lipid metabolism were also excluded. The mean (±SEM) age was 54 ± 2 years, and body mass index (body weight in kg/height in m^2) was 28.3 ± 2.5 kg/m^2.

Each subject was studied on two isocaloric diets (approximately 35 calories/kg): one contained 40% carbohydrate, 41% fat, and 19% protein and the other contained 60% carbohydrate, 21% fat, and 19% protein as percent of total calories. The P/S ratio on both diets was kept at 0.4. Diets were administered in a random fashion, each one was consumed for 15 days, and there was no break between dietary periods. Five patients began the study with the 60% carbohydrate diet, while three began with the 40% carbohydrate diet. Thus, each patient served as his or her own control. Composition of the diets and sample menus were described in detail previously.[13] Body weights were maintained within 1.0 kg of the baseline value throughout the study.

Blood was obtained after an overnight fast on days 13, 14, and 15 of each dietary period. In addition, blood was drawn on days 14 and 15 just before the noon meal. The meal was consumed over a 20-minute period, and blood samples were then taken hourly for the next six hours on days 14 and 15. Measurements were made of glucose, insulin, total TG, and cholesterol concentrations in plasma, and TG and cholesterol concentrations of lipoprotein fractions. The data reported here represent the means of these measurements.

Lipoproteins were separated by ultracentrifugation[18] into chylomicrons (chylo), very-low-density lipoproteins (VLDL, $d < 1.006$), low-density lipoproteins (LDL, $d = 1.006$–1.063), which may contain IDL in postprandial specimens, and high-density lipoproteins

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(HDL d > 1.063). The HDL was spun at d = 1.125 (40,000 rpm × 24–30 h with a Spinco 40.3 Rotor at 14–21 °C to separate HDL_2 and HDL_3. Glucose, insulin, triglyceride and cholesterol concentrations were determined as described previously.[13] The Wilcoxon matched-pair signed rank test was used for statistical analysis.[19]

RESULTS

The effects of the two diets on fasting glucose, insulin, total TG, total cholesterol, and lipoprotein TG and cholesterol are seen in Table 1. These data indicate that the fasting plasma total TG concentration was increased when subjects consumed the 60% carbohydrate diet, and that this was secondary to an increase in VLDL-TG concentration. No other significant changes were noted.

Postprandial glucose and insulin concentrations are given in the top and middle panels of Figure 1. These data indicate that the postprandial glucose concentration was not significantly increased on the 60% carbohydrate diet. On the other hand, mean plasma insulin levels were significantly higher at noon ($P < 0.02$), before eating lunch, and one ($P < 0.005$), five ($P < 0.005$), and six hours ($P < 0.02$) after eating the test meal containing 60% carbohydrate. In addition, the total integrated insulin response area during the six-hour postprandial period was significantly greater ($P < 0.01$) when subjects consumed the 60% carbohydrate diet.

Mean postprandial plasma TG concentrations are illustrated in the bottom panel of Figure 1 and indicate that ingestion of the 60% carbohydrate diet led to an elevation of mean plasma TG concentrations at 12 PM, before starting the noon meal, as well as one and six hours later. These differences were due to an increase in VLDL-TG concentration, since chylo-TG levels were the same on both diets. The mean total integrated TG response to the noon meal was also significantly increased ($P < 0.005$) on the 60% carbohydrate diet (3230 mg/dL · h) compared with the 40% (2462 mg/dL · h) carbohydrate diet. Again, this difference

Table 1. Fasting Plasma Concentration

Plasma Variable	40% Carbohydrate Diet	60% Carbohydrate Diet	P
Glucose (mg/dL)	95 ± 3	92 ± 2	NS
Insulin (μU/mL)	8 ± 1	9 ± 1	NS
Total TG (mg/dL)	238 ± 20	348 ± 36	<0.005
Chylo-TG (mg/dL)	34 ± 6	44 ± 13	NS
VLDL-TG (mg/dL)	147 ± 15	241 ± 26	<0.005
Total Cholesterol (mg/dL)	224 ± 12	223 ± 14	NS
LDL-Cholesterol (mg/dL)	144 ± 7	127 ± 17	NS
HDL-Cholesterol (mg/dL)	31 ± 2	32 ± 3	NS
HDL_2-Cholesterol (mg/dL)	14 ± 2	15 ± 2	NS
HDL_3-Cholesterol (mg/dL)	17 ± 2	17 ± 1	NS

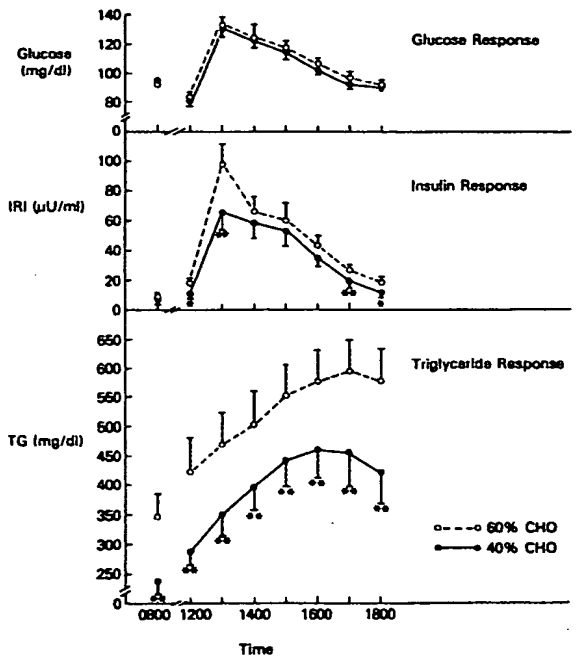

Fig. 1. Mean (±SEM) fasting and postprandial responses of glucose (top panel), insulin (middle panel), and triglyceride (lower panel) to isocaloric diets containing either 40% or 60% of total calories as carbohydrate. (* = $P < 0.05$; ** = $P < 0.01$)

was entirely related to higher VLDL-TG levels in the 60% carbohydrate diet.

Finally, there were no differences in postprandial responses of total cholesterol, LDL-cholesterol, HDL-cholesterol, HDL_2-cholesterol, or HDL_3-cholesterol to the two diets.

DISCUSSION

Changes in the relative proportion of dietary fat and carbohydrate can have profound effects on various aspects of carbohydrate and lipid metabolism. This ability to modulate plasma levels of hormones and substrates has obvious health-related implications. For example, there is evidence from prospective studies that fasting hypercholesterolemia[9] and hypertriglyceridemia,[6] postprandial hyperglycemia[7] and hyperinsulinemia,[4,5] and reduced fasting levels of HDL-cholesterol[8,9] are risk factors for the development of ASHD. Given these data, it seems reasonable to suggest that proposed manipulations of dietary fat and carbohydrate intake, aimed at reducing ASHD, be judged on the basis of their ability to improve these risk factors. If this criterion seems reasonable, it is difficult to understand current enthusiasm for isocaloric diets low in fat and high in carbohydrate. For example, there is evidence that such diets tend to produce both postprandial hyperglycemia and hyperinsulinemia,[1,2] and the cur-

rent study corroborates the deleterious effect of low-fat–high-carbohydrate diets on postprandial insulin responses. Thus, the institution of low-fat–high-carbohydrate diets can be expected to aggravate at least two risk factors involving carbohydrate metabolism that are thought to predispose an individual to the development of ASHD.

The effect of low-fat–high-carbohydrate diets on risk factors for ASHD related to lipid metabolism is more complicated. For example, although it has been apparent for some time that high-carbohydrate diets can lead to fasting hypertriglyceridemia,[1-14] it has been argued that postprandial hypertriglyceridemia is lower on such diets.[20] This was not true in our previous study of normal individuals,[13] and it is apparent from the data in Figure 1 that it also was not the case in the present study. Plasma TG concentrations were significantly higher at 8 AM, before breakfast, at 12 PM, before lunch, at 1 PM, one hour after lunch, and at 6 PM, just before dinner. Indeed, TG levels were *never* lower on the 60% carbohydrate diet. Fractionation of plasma lipoproteins indicated that the increase in both fasting and postprandial TG levels was due to an increase in VLDL-TG. In addition, postprandial chylo-TG concentrations were noted to be similar on the two diets. This seemed somewhat surprising in light of the reduced fat content of the 60% carbohydrate diet. However, the data in Table 1 indicate that fasting chylo-TG levels were somewhat higher on the 60% carbohydrate diet, thereby providing a plausible explanation for why an increase in dietery fat content did not lead to an increase in postprandial exogenous fat concentration. Finally, total plasma cholesterol, LDL-cholesterol, HDL-cholesterol, HDL_2-cholesterol, and HDL_3-cholesterol concentrations, both fasting and postprandial, were similar in both diets. Thus, it is apparent that there was some disadvantage, and no advantage, in terms of lipoprotein metabolism, to ingestion of a diet restricted in fat content (40% carbohydrate) but high in carbohydrate.

In conclusion, the evidence presented indicates that low-fat–high-carbohydrate diets accentuate known risk factors for ASHD in subjects who are already at risk. Indeed, the magnitude of the effect was greater in those patients with endogenous hypertriglyceridemia than in normal subjects. It is important to note, however, that the impact of variations in dietary carbohydrate may vary as a function of the kind of carbohydrate ingested. Thus, Lewis and associates[21] have indicated that tripling the amount of dietary fiber reversed the increase in VLDL-TG and decrease in HDL_2-cholesterol produced when an equally high carbohydrate diet (59% of total calories) containing more conventional foods was used. Additional support for the importance of the form of dietary carbohydrate in the regulation of lipid metabolism is the recent report by Reiser et al,[22] which emphasized the importance of dietary sucrose content in control of plasma lipid levels. It seems obvious that these issues should be clarified before advocating the general use of low-fat–high-carbohydrate diets in an effort to retard development of ASHD.

REFERENCES

1. Reaven GM, Olefsky JM: Increased plasma glucose and insulin response to high carbohydrate feeding in normal subjects. J Clin Endocrinol Metab 38:151–154, 1974
2. Ginsburg H, Olefsky JM, Kimmerling G, et al: Induction of hypertriglyceridemia by a low-fat diet. J Clin Endocrinol Metab 42:729–735, 1976
3. Gonen B, Patsch W, Kuisk I, et al: Effect of short-term feeding of a high carbohydrate diet on HDL subclasses in normal subjects. Metabolism 30:1125–1129, 1981
4. Pyorala K: Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. Diabetes Care 2:131–141, 1979
5. Ducimetiere P, Eschwege E, Papoz L, et al: Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary disease mortality in a middle-aged population. Diabetologia 19:205–210, 1980
6. Carlson LA, Bottiger LE, Ahfeldt PE: Risk factors for myocardial infarction in the Stockholm prospective study: a 14-year follow-up focusing on the role of plasma triglycerides and cholesterol. Acta Med Scand 206:351–360, 1979
7. Fuller JH, Shipley MJ, Rose C, et al: Coronary-heart disease risk and impaired glucose tolerance. Lancet 1:1373-1376, 1980
8. Miller GJ, Miller NE: Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet 1:16–19, 1975
9. Castelli WP, Doyle JT, Gordon T, et al: HDL cholesterol and other lipids in coronary heart disease. The comparative lipoprotein phenotyping study. Circulation 55:767–772, 1977
10. American Diabetes Association: Principles of nutrition and dietary recommendation for individuals with diabetes mellitus. Diabetes Care 2:520–523, 1979
11. Committee on Nutrition, American Heart Association: Circulation 58:762A–766A, 1978
12. Nutrition and Your Health—Dietary Guidelines for Americans. USDA-DHEW, Home and Garden Bulletin No. 232, 1980
13. Coulston AM, Liu GC, Reaven GM: Plasma glucose, insulin, and lipid responses to high carbohydrate-low fat diets in normal humans. Metabolism 32:52–56, 1983
14. Farquhar JW, Frank A, Gross RC, et al: Glucose, insulin, and triglyceride responses to high and low carbohydrate diets in man. J Clin Invest 45:1648–1656, 1966
15. Reaven GM, Lerner RL, Stern MP, et al: Role of insulin in endogenous hypertriglyceridemia. J Clin Invest 46:1756–1767, 1967
16. Gidez LI, Miller GJ, Burstin M, et al: Separation and quantitation of subclasses of human plasma high density lipoprotein by a simple precipitation process. J Lipid Res 23:1206–1222, 1982
17. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039–1057, 1979

18. Havel RJ, Eden HA, Bragdon JH: The distribution and chemical composition of untracentrifugally separated lipoproteins in human serum. J Clin Invest 34:1345–1353, 1955

19. Snedecor GW, Cochran WG: Statistical Methods. Ames, IA, Iowa State University Press, 1967

20. Schlierf G, Reinheimer W, Strossberg V: Diurnal patterns of plasma triglycerides and free fatty acids in normal subjects and in patients with endogenous (type IV) hyperlipoproteinemia. Nutr Metab 13:80–91, 1971

21. Lewis B, Katau M, Merkx I, et al: Towards an improved lipid-lowering diet: additive effects of changes in nutrient intake. Lancet 2:1310–1313, 1981

22. Reiser S, Bickard MC, Hallfrisch J, et al: Blood lipids and their distribution in lipoproteins in hyperinsulinemic subjects fed three different levels of sucrose. Am J Clin Nutr 111:1045–1057, 1981